59 *Sarcopodium*

Dongyou Liu and R.R.M. Paterson

CONTENTS

59.1 INTRODUCTION

59.1.1 Classification and Morphology

The genus *Sarcopodium* (obsolete synonyms: *Tricholeconium*, *Periolopsis*, *Actinostilbe*, *Kutilakesa*, and *Kutilakesopsis*) covers a group of plant-infecting fungi in the family Nectriaceae, order Hypocreales, class Sordariomycetes, subphylum Pezizomycotina, phylum Ascomycota, kingdom Fungi. Among the 12 recognized species in the genus, *Sarcopodium araliae*, *Sarcopodium circinatum* (the type species of the genus), *Sarcopodium oculorum*, and *Sarcopodium tortuosum* are relatively common plant pathogens; and *S. oculorum*, named after the infection site, has been shown to cause human infection [1–3].

On potato dextrose agar (PDA) at 25°C, *S. oculorum* colonies reach a diameter of 39–40 mm after 14 days. Initially, colonies are flat, mucoid, and cream colored but later become radially folded, brownish gray, and granulose due to the production of conidiomata. The colonies are grayish white toward the periphery with sparse aerial mycelium; the reverse is colorless to brown. On oatmeal agar (OA) and potato carrot agar (PCA) at 25°C, *S. oculorum* colonies grow more rapidly than on PDA, achieving a diameter of up to 48 and 45 mm, respectively, after 14 days. Colonies are granulose at the center and smooth toward the periphery, with whitish, soft cottony aerial mycelium and brownish gray submerged hyphae; the reverse is brownish gray and paler toward the periphery. On PDA at 37°C, *S. oculorum* colonies measure 14–15 mm in diameter after 20 days. Colonies are elevated and cerebriform, with abundant sporulation, but no conidiomata develops. *S. oculorum* does not grow at 40°C [3].

S. oculorum typically produces two types of conidia: one is derived from sporodochial conidiomata (cushion-like structures bearing numerous compact, short conidiophores, which produce the conidial mass) and the other is derived from undifferentiated hyphae. Sporodochia are superficial, solitary, gregarious or confluent, sessile, applanate to cupulate, or pulvinate, subhyaline to dark brown, setose, and up to 400 µm in diameter. Sterile hyphae (setae) form a frill at the margin of the sporodochium but are also interspersed with the conidiophores. Sterile hyphae (65 µm × 1.5–2.5 µm) are single or form small fascicles of 3–5 hyphae and are erect, unbranched or slightly branched toward the base, straight or flexuose, septate, subhyaline to dark brown, smooth walled, thin to slightly thick walled, and cylindrical, with obtuse apices. The conidiophores (up to 35 µm long) are well differentiated, straight or flexuose, subhyaline to pale brown, and smooth walled. They are irregularly branched, and each branch usually bears a single terminal group of slightly appressed conidiogenous cells. The conidiogenous cells (8–13 µm × 1–1.8 µm) are enteroblastic, monophialidic, terminal or lateral, hyaline to subhyaline, smooth walled, subcylindrical, and rarely intercalary with a cylindrical and lateral projection. These intercalary conidiogenous cells are predominantly found on undifferentiated hyphae. The conidia are aggregated in cream-colored slimy masses, which remain attached to the upper part of the conidioma and cover the entire surface. The individual conidia (1.2–3 µm × 0.8–1.5 µm) are subhyaline, aseptate, smooth and thin walled, ellipsoid, and navicular or slightly allantoid. They reach up to 5 µm and are cylindrical or allantoid when they emerge from undifferentiated hyphae [3].

Although *S. oculorum* and *S. circinatum* have sessile sporodochia, unbranched setae, and ellipsoid or cylindrical conidia, *S. circinatum* produces flexuous or circinate and verrucose setae up to 6 µm wide and larger (7–10 µm × 2 µm) conidia that are never navicular or allantoid. *S. oculorum* is differentiated from *S. tortuosum* by the latter's orange slimy conidial masses and branched setae. *S. oculorum* also resembles *Myrothecium* spp. and *Stephanonectria keithii* in the order Hypocreales (Ascomycota). However, the sporodochial

sterile hyphae in *Myrothecium* are hyaline to subhyaline and confined to the edge of the sporodochium, and in *S. keithii* they are absent. Moreover, the conidial masses are green in *Myrothecium* and brown in *S. keithii* [3].

59.1.2 Clinical Features

Sarcopodium spp. are commonly present in plants, dead herbaceous stems, and dead wood in many parts of the world, but not in soil, air, or animals.

The only reported clinical case of *Sarcopodium oculorum* infection in humans involved a 12 year-old Brazilian boy with a vernal conjunctivitis [3]. The patient presented with a pain in his right eye and felt the presence of a foreign body. After a 5 month treatment for keratoconjunctivitis with specific antiallergic drugs, topical dexamethasone and prednisone, the patient developed a corneal ulcer as a consequence of the allergic process, showing redness of the eye with inflammatory infiltrate and a suspicion of infection. Direct examination of Gram-stained mounts of the deep corneal scrapings revealed the presence of numerous septate and branched hyphae and a few ellipsoidal conidia. Culture of the corneal scrapings on Sabouraud dextrose agar at 25°C, 30°C, and 37°C for 4–5 days, yielded several colonies of a single, darkly pigmented fungus, which was verified as *Sarcopodium oculorum* on the basis of its distinct morphological, biochemical, and molecular features. The infection was resolved with natamycin and ketoconazole [3].

59.1.3 Diagnosis

The microscopic characteristics of *Sarcopodium oculorum* are examined under a light microscope upon preparation of wet mounts with lactic acid. Photomicrographs may also be obtained by scanning electron microscopy. To differentiate from other *Sarcopodium* species, a conidial suspension of *Sarcopodium oculorum* may be inoculated onto sterilized plant material since many plant pathogenic fungi show different morphological features on natural substrates than on laboratory culture media.

Sarcopodium is a hyphomycetous genus and is characterized by the presence of sporodochia with sterile, smooth or ornamented, often coiled pale brown setae arising from branched conidiophores and producing slimy conidia from phialides. The conidia are hyaline, aseptate, and fusiform to ellipsoid or cylindrical. The presence of numerous, dark sporodochia covered by a wet mass of cylindrical, hyaline conidia may be confused with the typical conidiomata (pycnidia) of *Phoma* or other similar coelomycetous fungi under a stereomicroscope. However, these structures are easily differentiated at high magnification. The pycnidia are closed spherical or obpyriform structures that are open only at the apical part by an ostiole. Additionally, the pycnidia possess a pseudoparenchymatous wall that is absent in the sporodochia. Conidiophores are formed inside the pycnidia and lining the internal cavity. Other coelomycetous fungi such as *Colletotrichum* develop acervular conidiomata (cup-shaped fruiting bodies) on natural substrates and turn sporodochial in vitro.

Given that culture-based methods for fungal diagnosis require up to 3 weeks of incubation, PCR amplification and sequencing analysis of rRNA intergenic spacer (ITS) regions provide a rapid and accurate approach for *S. oculorum* identification [4–6].

59.2 METHODS

59.2.1 Sample Preparation

Sarcopodium specimens are grown on inhibitory mold agar, modified Sabouraud agars, or PDA. On OA (30 g of oat flakes, 1 g of $MgSO_4 \cdot 7H_2O$, 1.5 g of KH_2PO_4, 15 g of agar, 1000 mL of tap water) and PCA (20 g of potatoes, 20 g of carrot, 18 g of agar, 1000 mL of tap water) may also be used.

After growth for 1–7 days on PDA slants, lysates are prepared from approximately 1 cm^2 of mycelia with IDI lysis kits (GeneOhm Sciences). Briefly, in a biological safety cabinet, mycelia are collected by scraping the slant with a sterile stick in 1 mL of sterile, molecular-grade H_2O. The material is transferred to a 2 mL screw-cap tube. The tubes are centrifuged for 1 min at 6000 × *g*. If the mycelia do not pellet, the material is contained with a pediatric blood serum filter (Porex Corp., Fairburn, GA). After removal of supernatant, the material is resuspended in 200 µL of IDI sample buffer and transferred to the lysis tube, which contains glass beads. Lysis tubes are vortexed on the highest setting for 5 min. The tubes are placed in a boiling water bath for 15 min. Tubes are centrifuged for 5 min at 16,000 × *g*. The supernatant is stored at –20°C until amplification [5].

59.2.2 Detection Procedures

Pounder et al. [5] described a real-time PCR with SYBR green DNA-binding dye and amplicon melting temperature analysis for fungal detection using pan-fungal primers ITS1 forward (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 reverse (5′-TCCTCCGCTTATTGATATGC-3′) [7]. The identity of the fungi is verified by subsequent sequencing analysis.

Procedure

1. PCR mixture is composed of 1× Lightcycler FastStart DNA Master Hybridization Probes mixture (Roche Applied Science) (containing deoxynucleoside triphosphates, FastStart *Taq* DNA polymerase, and 1 mM $MgCl_2$, additional $MgCl_2$ is added to a final concentration of 4.6 mM), 0.4 µM each of ITS1 forward and ITS4 reverse primers, 1× SYBR green (Molecular Probes), and 3 µL template DNA.

2. Thermal cycling parameters include 95°C for 10 min; 50 cycles of 95°C for 5 s, 60°C for 20 s, and 76°C for 30 s; and a final extension at 72°C for 2 min.
3. The quality of the amplicon is determined using the derivative of the melt analysis curve (55°C–99°C, 45 s hold at 55°C, 5 s/°C) using the RotorGene 3000 (Corbett Robotics, Inc).
4. The amplified product is purified for bidirectional sequencing using ExoSAP-IT (USB Corp). Five microliters of Big Dye Terminator Ready Reaction Mix v. 1.1 (Applied Biosystems) is added to 4 µL of each primer (0.8 pmol/µL) and 3 µL of purified PCR product. Cycle sequencing is performed with a 9700 thermal cycler (ABI), using 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Sequencing reaction products are passed through a Sephadex G-50 fine column to remove unincorporated dye terminators. Purified sequencing reaction products are run on an ABI Prism 3100 Genetic Analyzer with a 50 cm capillary array.
5. Sequences are analyzed with the SmartGene Integrated Database Network software version 3.2.3 vr. SmartGene is a web-based software and database system with reference sequences derived from the National Center for Biological Information (NCBI) GenBank repository.

Note. Sequence-based identifications are defined by percent identity: species, ≥99%; genus, 93%–99%; and inconclusive, ≤93%. For strains producing discrepant identification between the methods based on phenotypic characteristics and ITS sequence analysis, the D1-D2 region of the large-subunit rRNA gene is amplified with primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) and sequenced for species clarification (Leaw et al. 2006). AQ1

59.3 CONCLUSION

The genus *Sarcopodium* consists of 12 recognized fungal species that are commonly found in plants, dead herbaceous stems, and dead wood worldwide. Only one case of *Sarcopodium oculorum*-related vernal conjunctivitis has been reported recently [3]. Because many fungal species such as members of the *Phoma* and *Colletotrichum* genera may also cause keratomycosis, there is a need to correctly identify them for treatment purpose. The fact that some of these fungi sometimes fail to sporulate in culture often delays the result availability based on morphological and biochemical criteria. Use of PCR and sequencing offers a rapid and precise means of determining the identity of *Sarcopodium* and morphologically similar fungi.

REFERENCES

1. Sutton, B. C. 1981. *Sarcopodium* and its synonyms. *Trans Br Mycol Soc*. 76:97–102.
2. Watanabe, T. 1993. *Sarcopodium araliae* sp. nov. on root of *Aralia elata* from Japan. *Mycologia*. 85:520–526.
3. Guarro, J. et al., Corneal ulcer caused by the new fungal species Sarcopodium oculorum. *J Clin Microbiol*. 2002;40(8):3071–3075.
4. Leaw, S. N. et al., Identification of medically important yeast species by sequence analysis of the internal transcribed spacer regions. *J Clin Microbiol*. 2006;44(3):693–699.
5. Pounder, J. I et al. Discovering potential pathogens among fungi identified as nonsporulating molds. *J Clin Microbiol*. 2007;45(2):568–571.
6. Bagyalakshmi, R. et al. Newer emerging pathogens of ocular non-sporulating molds (NSM) identified by polymerase chain reaction (PCR)-based DNA sequencing technique targeting internal transcribed spacer (ITS) region. *Curr Eye Res*. 2008;33(2):139–147.
7. White, T. J. et al. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Snisky, and T. J. White (eds.), *PCR Protocols*: *A Guide to Methods and Applications*. Academic Press, San Diego, CA, pp. 315–322.

AUTHOR QUERY

[AQ1] Reference citation Leaw et al. (2006) is not provided in the References list. Please check.